SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES

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April 4, 2005

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A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

May 2005

Abstract

Silver nanoparticles may be prepared in aqueous solution by reducing silver nitrate with borohydride in the presence of sodium citrate and 4-4-(phenylphosphinidene) bis-(benzene sulfonic acid) (BSPP). Variation of the reaction conditions produces nanoparticles of different sizes, shapes, and chemical properties. For instance, the resistance to oxidation in the presence of cyanide can be increased by synthesizing nanoparticles using a high concentration of citrate. The differing chemical properties also show different results when using silver nanoparticles as a catalyst for the reduction of dichlorofluorescein. An induction time for the reduction reaction to begin was longer when nanoparticles were used that had been synthesized with a high concentration of BSPP. Finally, the surface effects of sulfur containing molecules on silver nanoparticles differ depending upon nanoparticle preparation. For example, silver nanoparticle solutions that were prepared with a high concentration of citrate showed a smaller shift in the λ_{max} than those with a low concentration of citrate with the addition of L-cysteine.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to extend my gratitude to the Department of Chemistry and Biochemistry for its support, financially and otherwise. Without the assistance of the faculty, staff, and students this project could not have been completed. In particular, I would like to thank Sheila Chapin for clarifying paperwork and policies throughout this process; George Lee, my brother, who worked beside me in the lab and provided encouragement and healthy sibling rivalry when needed; and Josh Klynstra, another student that worked in our research lab, for providing information on the projects he completed.

I would also like to thank my committee for guiding me through this program. I would like to thank Dr. Simpson for his interest and enthusiasm for chemistry that inspired me to think about the fascinating aspects of my research; Dr. Trainor for allowing me to use his lab and always answering any questions that I came to him with; and Dr. Howard for introducing me to how interesting inorganic chemistry is and always helping me with my numerous questions. Finally, I would like to thank Dr. Stolzberg, who has been my mentor, teacher, and friend throughout both my undergraduate and graduate programs. Dr. Stolzberg pushed me to limits I never thought I could achieve and showed me how to better myself as both a chemist and a person. I am thankful for having the opportunity to work with such an incredible person. I would like to thank him and all of my committee for their support and wisdom throughout my education here at the University of Alaska Fairbanks.

Chapter 1

Introduction

Nonspherical silver nanoparticles are formed by chemical reduction of Ag^+ (aq), followed by photochemical transformation in the presence of 4-4-(phenylphosphinidine) bis-(benzene sulfonic acid) (BSPP), and tri-sodium citrate.[1] Variations in initial concentrations of BSPP, citrate, and the reducing agent borohydride result in solutions with dramatically different color and spectra which indicate different sizes and shapes of silver nanoparticles.

Aqueous suspensions of noble metal nanoparticles from ten to one hundred nanometers in size are unstable with respect to the agglomeration to bigger particles. The agglomeration can be prevented if the metal particles are surrounded by an electrical double layer providing electrostatic stabilization.[2] The result is stable nanoparticle suspensions with unique optical properties that can be used as part of a sensor system.[3-11]

The optical properties include unique absorbance bands that make noble metal nanoparticles useful as optical biosensors[3], immunoassays[4], in the monitoring of diseases[5], proteomics[6, 7], and the detection of ions in solution[8]. Recent research topics have included controlling the size and shape of nanoparticles[1, 9, 10, 11]; characterization of the shape of nanoparticles in relation to their plasmon resonance band[12]; stabilization of nanoparticles using carboxylate functional groups[13]; and surface modification of nanoparticles by adsorbate molecules such as dialkyl disulfides, imidazole, and mercaptoundecanoic acid onto nanoparticle surfaces[14, 15, 16].

Jiang et al[17] found that silver colloids could be prepared without adding any other stabilizers if carbon disulfide was added before reduction of silver nitrate. The silver nanoparticles were prepared by adding aqueous silver nitrate to CS_2 (aq), followed by KBH_4 reduction. The 395 nm absorption band characteristic of spherical silver colloids was observed. There was no investigation of the effect of CS_2 on pre-prepared silver nanoparticles or solutions with more than one stabilizing agent after reduction has occurred.

Haes and Van Duyne[18, 19] investigated the use of triangular silver nanoparticles as a bioassay for the biotin-streptavidin conjugate system. Biotin is a sulfur containing biomolecule that has a high affinity for the tetrameric protein streptavidin. Biotin functionalized with 3,6-dioxaoctanediamine was attached to triangular silver nanoparticles followed by the addition of streptavidin. Surface enhanced Raman spectroscopy was used to evaluate the shift in the plasmon resonance band of triangular silver nanoparticles following the addition of streptavidin. They found that triangular silver nanoparticles could be used as a sensitive bioassay for the biotin-streptavidin conjugate system with a limit of detection of 1 picomolar. The only nanoparticles used were triangular which raised the question of whether a different shaped silver nanoparticle could be used to produce a more sensitive bioassay.

Mulvaney, Linnert, and Henglein[20] investigated the effect of nucleophilic reagents on surface reactions. Silver colloids were exposed to CN^- or SH^- ions, followed by addition of organic electrophiles. The organic electron acceptors were used in place of oxygen in the process of oxidation. The changes in the shape of the surface plasmon

absorption bands were observed at various times after the addition of the acceptors. The oxidation of the silver colloids was in fact facilitated by using an organic electron acceptor in the presence of a nucleophile. It was noted that if the electron acceptor was added in the absence of the nucleophile, oxidation of the silver colloid would not occur, posing the question of whether or not oxidation will always occur in the presence of a nucleophile. Do different sizes and shapes of nanoparticles show a higher resistance to oxidation in the presence of a nucleophile such as CN^-?

The goals of present research at the University of Alaska Fairbanks are to evaluate the chemical and optical properties of nanoparticles synthesized using varying concentrations of reactants. It was hypothesized that by varying the reaction conditions, nanoparticles with varying sizes and shapes would be synthesized that would behave differently in a variety of chemical situations. This was done by using a three level factorial design varying the reaction conditions of citrate, BSPP, and borohydride. Each preparation was evaluated on how it behaved in experiments involving the addition of sulfur containing molecules, the reduction of dichlorofluorescein in the presence of silver nanoparticles, and the oxidation of the silver nanoparticles in the presence of cyanide.

Chapter 2

Experimental Procedures

2.1 Preparation of Silver Nanoparticles

Nanoparticle solutions were synthesized in acid cleaned 150 ml Erlenmeyer flasks with 2M nitric acid. Reagent grade silver nitrate (Thorn Smith Laboratories), sodium citrate (Aldrich), 4-4-(phenylphosphinidine) bis-(benzene sulfonic acid) (BSPP) (Aldrich), and sodium borohydride (Aldrich) were used in the syntheses. There were four separate syntheses over the course of three semesters, which were slightly different from one another. The first set of syntheses were done in the spring of 2002 (Table 1). The order of addition involved adding an aliquot of citrate to 1.0×10^{-4}M silver nitrate, followed by the addition of BSPP, and finally borohydride. Volumetric manipulations were done using a variable volume micropipette (Ranin, EDP2).

Table 1: Preparation of Silver Nanoparticles in the Spring of 2002

Standard Order	[Citrate] (μM)	[BSPP] (μM)	[Borohydride] (μM)
1	100	50	500
2	100	50	1000
3	100	100	500
4	100	100	1000
5	200	50	500
6	200	50	1000
7	200	100	500
8	200	100	1000

All solutions were placed two inches beneath two 40-Watt shop lights where they were left for three days during which photo transformation occurred at room temperature. UV-visible spectra were taken using a Hewlitt Packard 8453A UV-visible spectrometer. The CS_2 experiments were done using the nanoparticles from this preparation.

In the fall of 2002, the reagents remained the same, however, the design changed slightly (Table 2).

Table 2: Preparation of Silver Nanoparticles in the Fall of 2002.

Standard Order	[Citrate] (μM)	[BSPP] (μM)	[Borohydride] (μM)
1	100	50	500
2	100	100	500
3	200	50	500
4	200	100	500
5	100	50	1000
6	100	100	1000
7	200	50	1000
8	200	100	1000

The experiments that involved using this design included the dichlorofluorescein experiment, the central composite design experiment, and experiment testing how easily oxidized the silver nanoparticles were in the presence of cyanide. These solutions were also photo transformed under two 40-Watt shop light.

In the fall of 2003, the design was changed to include a full spectrum light source in addition to the shop light, as well as the order of addition of borohydride and BSPP. There were two slightly different designs used during this time. One was prepared on

September 19, 2003 (Table 3) and the other was prepared on November 6, 2003 (Table 4). The same reagents were used as described above.

Table 3: Preparation of Silver Nanoparticles on September 19, 2003.

Standard Order	[Citrate] (μM)	[BSPP] (μM)	[$NaBH_4$] (μM)	Light Source	Order of Addition
1	100	50	500	shop light	$NaBH_4$ first
2	200	50	500	full spectrum	BSPP first
3	100	100	500	full spectrum	$NaBH_4$ first
4	200	100	500	shop light	BSPP first
5	100	50	1000	shop light	BSPP first
6	200	50	1000	full spectrum	$NaBH_4$ first
7	100	100	1000	full spectrum	BSPP first
8	200	100	1000	shop light	$NaBH_4$ first
2*	200	50	1000	full spectrum	BSPP first

Standard Order 2* was done with the addition of 1000μM rather than 500μM sodium borohydride. The solutions were allowed to photo transform for four days and then removed from the light source and placed in the refrigerator at 5°C. These solutions were used for the preliminary studies using L-cysteine and 1-dodecanethiol. The second set of silver nanoparticles synthesized on November 6, 2003 had the following design (Table 4).

Table 4: Preparation of Silver Nanoparticles on November 6, 2003.

Standard Order	[Citrate] (μM)	[BSPP] (μM)	[$NaBH_4$] (μM)	Light Source	Order of Addition
1	100	50	500	full spectrum	$NaBH_4$ first
2	200	50	500	shop light	BSPP first
3	100	100	500	shop light	$NaBH_4$ first
4	200	100	500	full spectrum	BSPP first
5	100	50	1000	full spectrum	BSPP first
6	200	50	1000	shop light	$NaBH_4$ first
7	100	100	1000	shop light	BSPP first
8	200	100	1000	full spectrum	$NaBH_4$ first

The preparation of these silver nanoparticles is nearly identical to those synthesized on September 19 with only the light source changed. These solutions were also photo transformed for four days and then stored in the refrigerator. These solutions were used for sizing experiments.

2.2 Preliminary Studies on Surface Effects using Sulfur Containing Molecules

Cysteine

There were four similar experiments done using L-cysteine. The first experiment done was to add 0%, 25%, 50%, 75%, and 100% molar ratio of cysteine to silver into each of the silver nanoparticle solutions. This was done by taking 2 ml aliquots of Standard Orders 1 and 4 (Table 3) and adding 0, 25, 50, 75, and 100μL of 2×10^{-3}M L-cysteine. Duplicates were made of each solution. One was placed in the dark after addition of L-cysteine, while the other set was put under the shop light. UV-visible spectra were taken at 10 minutes, 30 minutes, 24 and 42 hours after addition.

The second experiment was done in the same way, with a molar ratio of cysteine to silver to 0, 5, 10, 15, and 20% into 2 ml of Standard Orders 1 and 4. UV-visible spectra were taken ten and thirty minutes after addition of L-cysteine.

The third experiment was done similar, with a molar ratio of 0, 1, 2, 3, 4, and 5% cysteine to silver. This was done by taking 2 ml aliquots of Standard Orders 1 and 4 and adding 10, 20, 30, 40, and 50μL of 2×10^{-4}M L-cysteine. UV-visible spectra were taken 10 minutes and 1 hour after the addition of L-cysteine.

The fourth experiment done using L-cysteine was a photometric titration using each of the solutions described in table 3. This was done by adding 10μL increments of 2×10^{-5}M L-cysteine to 2-ml of each solutions over a range of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, and 5.0% molar ratio cysteine to silver. UV-visible spectra were taken 10 minutes after the addition of each L-cysteine aliquot.

1-Dodecanethiol

The next sulfur containing compound used was 1-dodecanethiol. Because dodecanethiol is insoluble in water experiments were first done using hexane and later methanol as the solvent. Standard Orders 6 and 7 (Table 3) were selected for the preliminary studies with hexane as the solvent. The dodecanethiol was expected to enter the aqueous phase and interact with the silver. A 5 ml aliquot of 1.0×10^{-6}M dodecanethiol in hexane was added to 5 ml of 1.0×10^{-4}M silver nanoparticle solution. This gave a molar ratio of 1% dodecanethiol to silver. The second experiment done using hexane as the solvent involved a three factorial design that included the presence of dodecanethiol, oxygenated versus deoxygenated, and two different silver preparations (Table 5). The "deoxygenated" solutions were purged with N_2(g).

Table 5: Preliminary Experiment Using Dodecanethiol.

Standard Order	Molar ratio of Dodecanethiol	Deoxygenated vs. Oxygenated	Silver Preparation (table 3)
1	0%	Deoxygenated	SO7
2	1%	Deoxygenated	SO7
3	0%	Oxygenated	SO7
4	1%	Oxygenated	SO7
5	0%	Deoxygenated	SO6
6	1%	Deoxygenated	SO6
7	0%	Oxygenated	SO6
8	1%	Oxygenated	SO6

The next solvent used to dissolve dodecanethiol was methanol. An experiment similar to the one described using hexane was designed. Dodecanethiol was present at two nonzero concentrations (Table 6). UV-visible spectra were taken at 30 minutes, 1 hour, and 24 hours after addition of dodecanethiol.

Table 6: Second Experiment using Dodecanethiol.

Standard Order	Molar ratio of Dodecanethiol	Deoxygenated vs. Oxygenated	Silver Preparation (Table 3)
1	1	Deoxygenated	SO6
2	3	Deoxygenated	SO6
3	1	Oxygenated	SO6
4	3	Oxygenated	SO6
5	1	Deoxygenated	SO7
6	3	Deoxygenated	SO7
7	1	Oxygenated	SO7
8	3	Oxygenated	SO7

Carbon Disulfide

There were two experiments done using carbon disulfide. The first experiment involved adding 100μL of 0.0040M CS_2 into 10 ml for a final concentration of 40μM CS_2 to month old silver nanoparticle solutions (Table 1). The second experiment increased the concentration of CS_2 by adding 500μL of 0.050M CS_2 to 10 ml for a final concentration of 250μM CS_2 to month old silver nanoparticle solutions. In addition 500μL of 0.05M CS_2 for a final concentration of 250μM CS_2 was added to freshly prepared silver nanoparticle solutions. UV-visible spectra were taken over a course of 2 days.

2.3 Oxidation of Silver Nanoparticles in the presence of Cyanide

The silver nanoparticles used in the cyanide experiment were those synthesized with the central composite design (Table 7). This experiment was done to explore the differences in how easily oxidized the different preparations of silver nanoparticles were in the presence of cyanide. This procedure involved adding a 10μL aliquot of 0.010M cyanide to 3 ml of each of the eight silver nanoparticle solutions. UV-visible spectra were then taken over a span of three days.

2.4 Catalytic Properties of Silver Nanoparticles

Catalytic properties of the silver nanoparticles were explored by the reduction of dichlorofluorescein using silver nanoparticles as a catalyst and borohydride as a reducing agent. For this procedure, 10 ml of 1.0×10^{-4}M silver nanoparticle solutions (Table 2) were added to 100 ml of 1.0×10^{-6}M dichlorofluorescein, followed by 10 ml of 0.015M sodium borohydride. The absorbance was measured at 402nm and 502nm every thirty seconds for twenty minutes on a HP8453A UV-Visible Spectrometer.

2.5 Central Composite Design

The central composite design used a preparation containing 100μM citrate, 100μM BSPP, and 500μM borohydride as a center point. The concentration of reactants for the other solutions was varied around this preparation. A total of sixteen solutions were prepared during this experiment (Table 7).

Table 7: Preparation of Central Composite Design Experiments.

Standard Order	[citrate] (μM)	[BSPP] (μM)	[borohydride] (μM)
1	70	85	350
2	130	85	350
3	70	115	350
4	130	115	350
5	70	85	650
6	130	85	650
7	70	115	650
8	130	115	650
9	50	100	500
10	150	100	500
11	100	75	500
12	100	125	500
13	100	100	248
14	100	100	752
15	100	100	500
16	100	100	500

2.6 Nanoparticle Sizing

Silver nanoparticles were prepared using the design from November 6, 2003 (Table 4). The reagents were combined and the standard order solutions were put under the full spectrum light. The solutions were removed from the light source at 9, 10, and 11 hours and their size was measured using the Brookhaven 90 Plus instrument. The Brookhaven 90 Plus instrument is a dynamic light scattering instrument that uses a time auto correlation to measure diffusion coefficients.[21] The diffusion coefficient is related to the diameter of the particle; this relation allows for the determination of the nanoparticle diameter size.

Chapter 3

Results and Discussion

3.1 Preparation of Silver Nanoparticles

After the photo transformation of the silver nanoparticle solutions, the color had changed from yellow to a range of various colors (Figure 1).

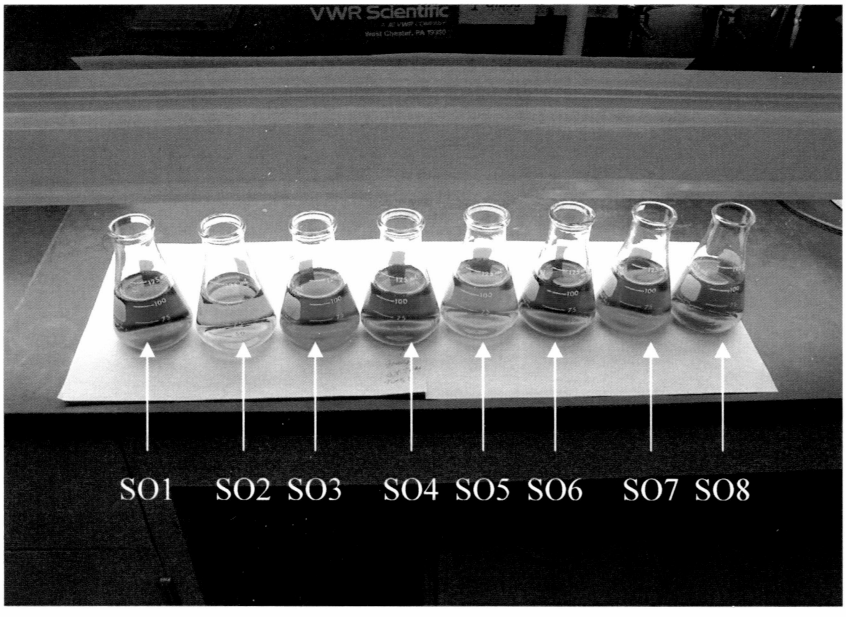

Figure 1: Silver Nanoparticles Synthesized from Table 2.

Figure 1 shows the standard order solutions 72 hours after the photo transformation process. The varying colors indicate that the size and shape of the silver nanoparticles differ depending upon the concentration of the reagents.[13] It is interesting

to note that those solutions that have a green tint to the color all have a high concentration of borohydride. It appears as though all of the reactants have an effect on the development of the nanoparticles. The various nanoparticle solutions had different absorbance bands with different peak wavelengths (Figures 2 and 3, Table 8).

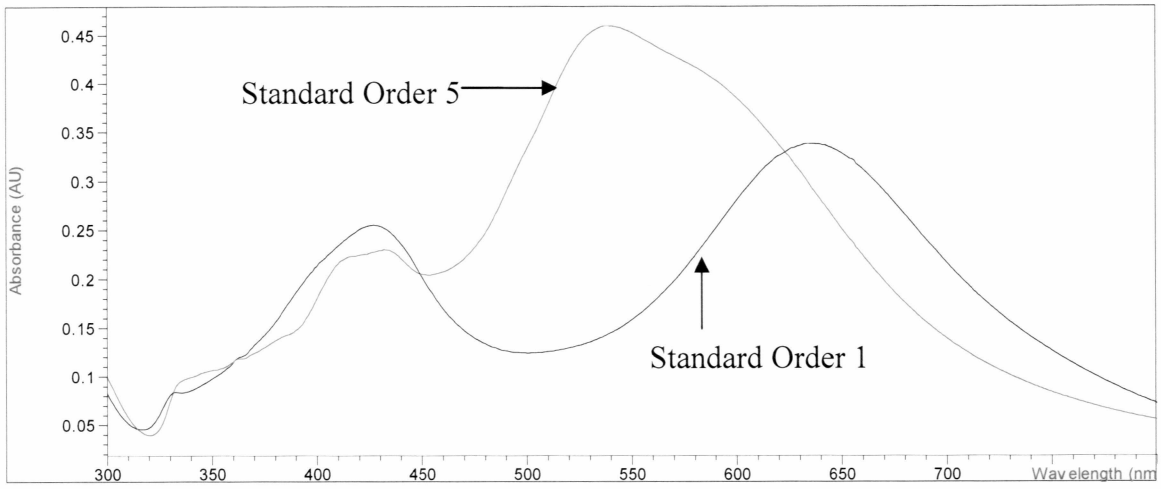

Figure 2: Standard Order 1 and Standard Order 5 72 hours after Photo Transformation.

Figure 2 shows Standard Orders 1 and 5. Standard Order 1 had a low concentration of citrate, BSPP, and borohydride. Standard Order 5 had a high concentration of citrate and low concentrations of BSPP and borohydride. The two spectra have different absorbance bands. Standard Order 1 had two distinct peaks while Standard Order 5 had one distinct peak, with a smaller peak not well resolved. This indicates that the different preparations of silver nanoparticles produced nanoparticles with differing properties.

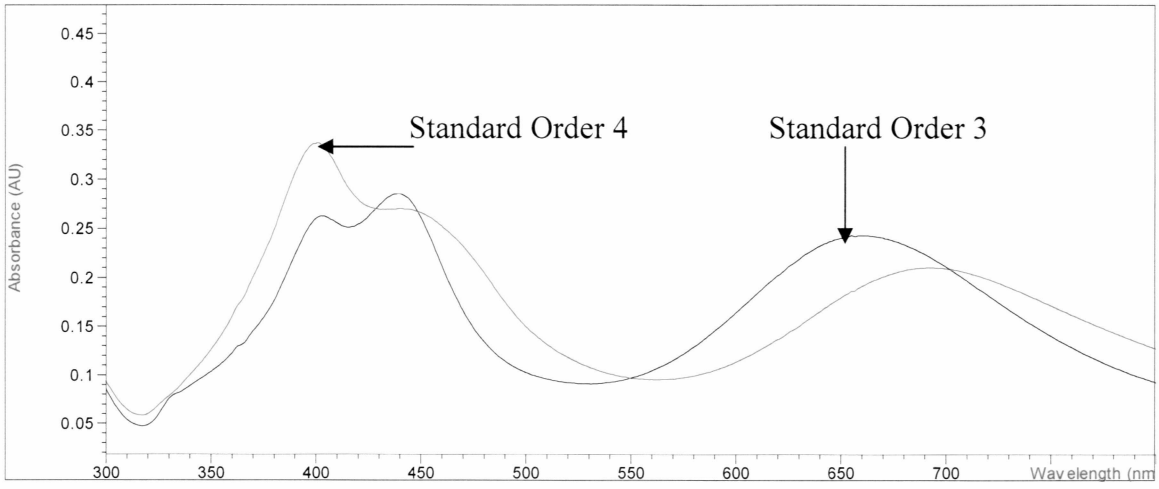

Figure 3: Standard Order 3 and Standard Order 4 72 hours after photo transformation.

Figure 3 shows Standard Orders 3 and 4. Standard Order 3 had low concentrations of citrate and borohydride and a high concentration of BSPP. Standard Order 4 had a low concentration of citrate and high concentrations of BSPP and borohydride. These two spectra also show that the different concentration of reagents produced different nanoparticles.

There were four different syntheses experiments done over the course of two years that showed slightly different λ_{max} and maximum absorbance values. Some preparations had two distinct absorbance bands which are identified together in Tables 8-11 .

Table 8: Peak Wavelength of Standard Order Solutions from Table 1.

Standard Order	[Borohydride] (μM)	[BSPP] (μM)	[Citrate] (μM)	λ_{max} (nm)	A_{max} (AU)
1	500	50	100	427, 634	0.255, 0.338
2	1000	50	100	466, 589	0.363, 0.244
3	500	100	100	439, 655	0.285, 0.243
4	1000	100	100	401, 693	0.337, 0.210
5	500	50	200	539	0.459
6	1000	50	200	517	0.415
7	500	100	200	491, 589	0.346, 0.268
8	1000	100	200	435, 519	0.269, 0.531

Table 9: Peak Wavelength of Standard Order Solutions from Table 2.

Standard Order	[BSPP] (μM)	[Citrate] (μM)	[Borohydride] (μM)	λ_{max} (nm)	A_{max} (AU)
1	50	100	500	642	0.719
2	100	100	500	464, 632	0.437, 0.531
3	50	200	500	491	0.619
4	100	200	500	621	0.710
5	50	100	1000	633	0.581
6	100	100	1000	432, 604	0.363, 0.373
7	50	200	1000	605	0.537
8	100	200	1000	608	0.630

Table 10: Peak Wavelength of Standard Order Solutions from Table 3.

Standard Order	[Citrate] (μM)	[BSPP] (μM)	[$NaBH_4$] (μM)	λ_{max} (nm)	A_{max} (AU)
1	100	50	500	440, 659	0.705, 0.698
2	200	50	500	632	1.15
3	100	100	500	444, 662	0.520, 0.401
4	200	100	500	580	1.23
5	100	50	1000	612	1.01
6	200	50	1000	450, 602	0.679, 1.12
7	100	100	1000	662	1.06
8	200	100	1000	612	0.993
2*	200	50	1000	624	1.14

Table 10 shows results from an experiment (Table 3) in which the order of addition and light source were explored. Standard orders 2, 4, 5, 7, and 2* all had BSPP added before the borohydride, while standard orders 1, 3, 6, and 8 had borohydride added before BSPP. Standard orders 1, 4, 5, and 8 were put under the shop light, while standard orders 2, 3, 6, 7, and 2* were put under the full spectrum light. The light source did not appear to have an effect on the synthesis of the silver nanoparticles. The order of addition had an effect on the appearance of the nanoparticle solutions; those solutions where borohydride were added first had a cloudy appearance, while those where BSPP was added first were clear.

Table 11: Peak Wavelength of Standard Order Solutions from Table 4.

Standard Order	[Citrate] (μM)	[BSPP] (μM)	[$NaBH_4$] (μM)	λ_{max} (nm)	A_{max} (AU)
1	100	50	500	398, 626	0.535, 0.631
2	200	50	500	617	1.62
3	100	100	500	413, 578	0.369, 0.470
4	200	100	500	622	1.34
5	100	50	1000	640	1.08
6	200	50	1000	444, 609	0.469, 1.03
7	100	100	1000	396, 594	0.821, 0.560
8	200	100	1000	448, 624	0.492, 1.02

3.2 Preliminary Studies on Surface Effects using Sulfur Containing Molecules

Silver nanoparticles have unique optical properties that make them likely candidates for use as part of a bioassay. Much of the research being done involves the use of one particular synthesis of nanoparticle as part of a biosensor.[18, 19] Different preparations of silver nanoparticles produced differing sizes and shapes that in turn have different chemical properties. The surface effects of sulfur containing molecules were investigated using nanoparticles synthesized with different preparations.

L-Cysteine

L-cysteine was the first sulfur containing molecule used to investigate the surface interactions with silver nanoparticles. The initial experiment done using cysteine involved selecting two separate silver preparations, standard order 1 and standard order 4 (Table 3), and adding the molar ratio of 0, 25, 50, 75, and 100% L-cysteine to silver.

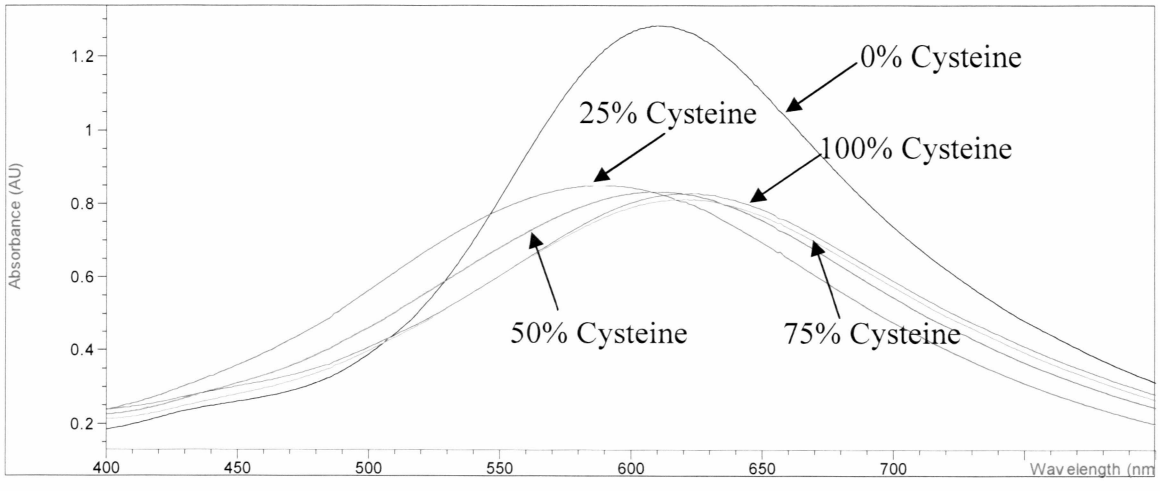

Figure 4: Spectra of Standard Order 4 30 minutes after L-Cysteine addition.

After the initial addition of cysteine to the silver nanoparticle solution, the absorbance band at lambda max decreased and shifted toward the blue (Figure 4). After the subsequent additions, the absorbance bands at lambda max shifted toward the red. There was a large change in the spectrum from 0 to 25 percent molar cysteine, with very small changes as the concentration of cysteine increased. It became apparent that there was a surface interaction occurring between the silver nanoparticles and the cysteine. The cysteine saturated the silver nanoparticle surface area at twenty five percent.

When the molar ratio was decreased to 0, 5, 10, 15, and 20% molar ratio cysteine to silver, a similar result was found. At the addition of 5% cysteine, there was a large decrease in the spectrum with a blue shift in the absorbance band at lambda max. The addition of 10, 15, and 20% cysteine showed a much smaller change in the spectra (Figure 5).

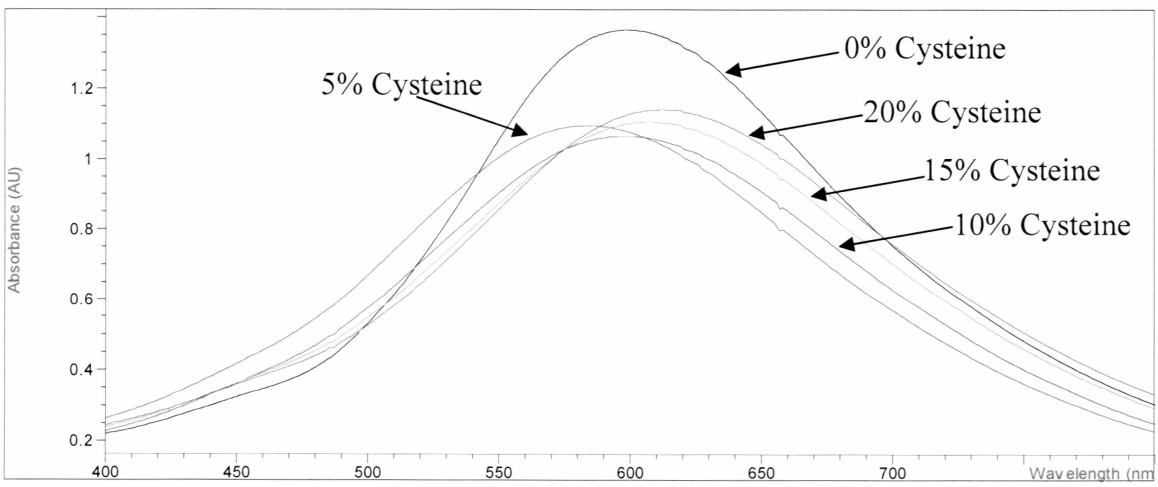

Figure 5: Spectra of Standard Order 4 30 minutes after L-Cysteine addition.

It appears that with the addition of smaller molar ratios of cysteine there was a smaller change in absorbance. An interesting phenomenon was observed when the absorbance at lambda max initially decreased and a blue-shift was observed after five percent cysteine addition. The absorbance continued to decrease and a red-shift was observed after the addition of 10% cysteine. When 15 and 20% cysteine was added, the absorbance actually increased and shifted toward the red. It is evident that a surface interaction is occurring at the small concentration of 5%.

Due to this observation, the next experiment reduced the molar ratio of cysteine to 0, 1, 2, 3, 4, and 5%, which showed results consistent with the interaction of cysteine with the calculated concentration of silver on the surface of nanoparticles. There is a significant decrease in absorbance after the addition of 1 percent cysteine, while the additions of 2, 3, 4, and 5% had smaller decreases in absorbance (Figure 6).

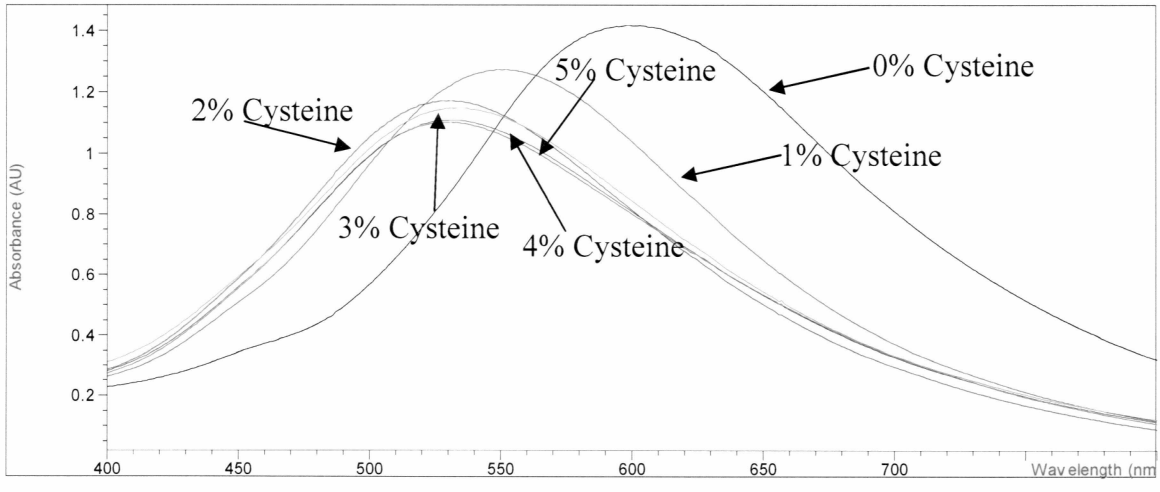

Figure 6: Spectra of Standard Order 4 30 minutes after L-Cysteine addition.

At this small range, the absorbance at lambda max decreased and shifted toward the blue with every addition of cysteine. This indicates that there is a high sensitivity of the silver nanoparticles to L-Cysteine.

Further exploration of this property was done by performing a photometric titration. The results showed different results for each of the standard order solutions (Figures 7 and 8).

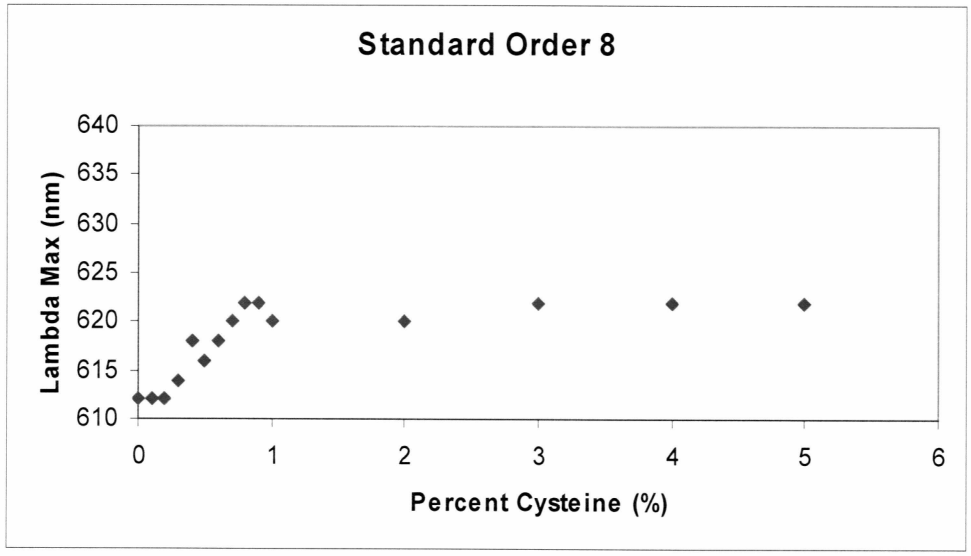

Figure 7: Molar Ratio of Cysteine added versus λ_{max} using Standard Order 8.

Figure 5 shows that there was a maximum shift of 10 nm toward the red, at 0.8% molar ratio cysteine added. This is a different result than that of Standard Order 3 (Figure 8).

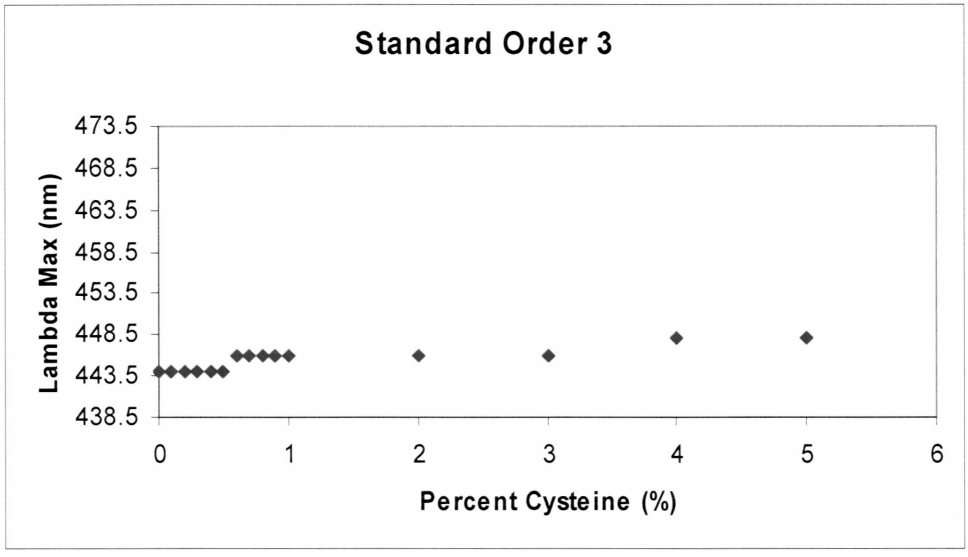

Figure 8: Molar Ratio of Cysteine added versus λ_{max} using Standard Order 3.

There was a total shift of 4 nm toward the red, which is a smaller result than that observed with standard order 8. This shift indicates that the surface interaction of the cysteine to the silver is different for the different preparations. Standard order 7 showed a different result from both standard order 8 and 3 (Figure 9).

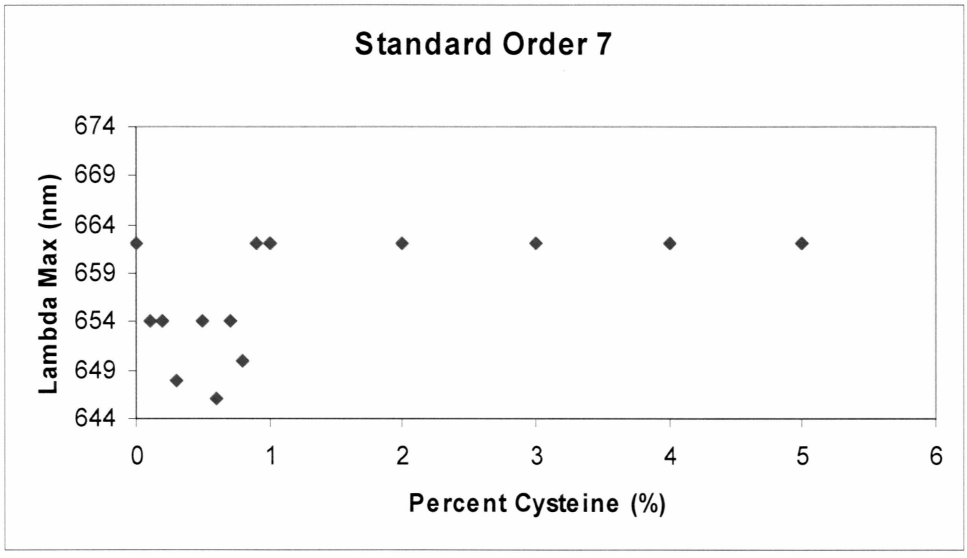

Figure 9: Molar Ratio of Cysteine added versus λ_{max} using Standard Order 7.

There was no net change of λ_{max} with the addition of cysteine. There was a small shift toward the blue after to addition of 0.3 and 0.8 percent cysteine. However, λ_{max} was the same at 5 percent cysteine and 0 percent cysteine, indicating no net change. Standard Orders 2, 2*, 5, and 6 showed similar results with a small change in λ_{max}.

The calculated value of molar percent silver atoms on the surface of silver nanoparticles ranges from approximately 3 to 7%. The range of molar ratio concentrations of cysteine falls within this range, the absorbance spectra changed as more cysteine was added to solution, binding to the silver atoms on the surface of the nanoparticles, indicating that the cysteine was continuing to bind to the nanoparticles. Experiments done with the concentration range above 5%, showed one large change in absorbance, and then very small changes. This large initial shift indicates that all of the

silver atoms on the surface of the nanoparticle were bound to from the initial addition of cysteine.

There continued to be a shift in the wavelength at lambda max, as well as a shift in absorbance over time. However, the reaction that occurred was slow and not dramatic (Figure 10).

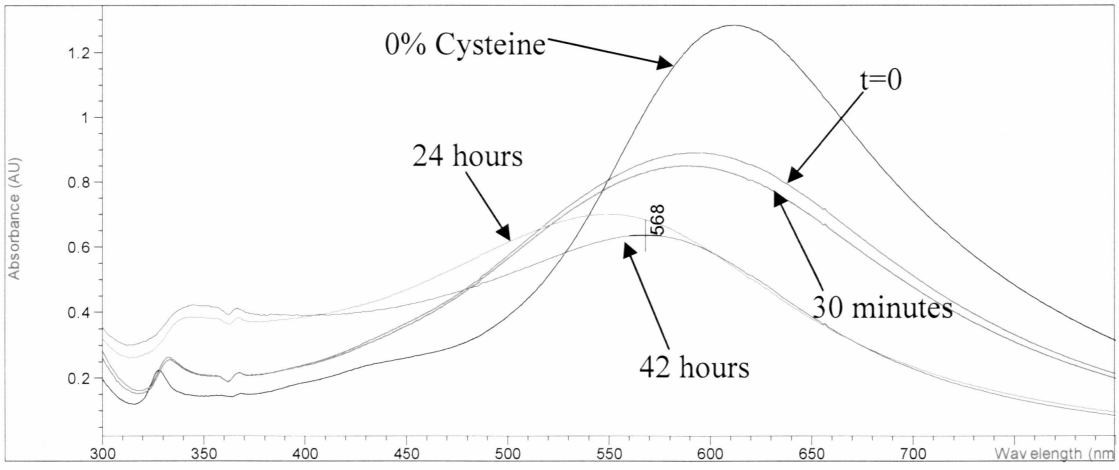

Figure 10: Standard Order 4 over Time after Cysteine Addition.

Figure 10 shows Standard Order 4 after the addition of 25% cysteine. There is a small decrease in absorbance over time as well as a shift toward the blue after the initial addition of cysteine. When cysteine is added to the silver nanoparticle solutions, the sulfur on the cysteine molecule has a high affinity for the silver atoms on the nanoparticle.

Preliminary Studies Using 1-Dodecanethiol

The next sulfur containing molecule used with the silver nanoparticle solutions was 1-dodecanethiol. This particular compound is insoluble in water therefore, it was important to find a solvent to dissolve the dodecanethiol that would work well with the silver nanoparticle solutions. The first solvent used was hexane, which is nonpolar and therefore insoluble in water. The first experiment done involved the addition of 1% molar ratio dodecanethiol in hexane to silver using standard order 7.

A second experiment involved deoxygenating the silver nanoparticle solutions containing both the organic and aqueous layers (table 5). Two hexane solutions containing 1-dodecanethiol and two containing no 1-dodecanethiol were deoxygenated by purging with $N_2(g)$ before adding them to the silver nanoparticle solutions. When this was done, the silver nanoparticle solutions were nearly completely decolorized (Figure 11).

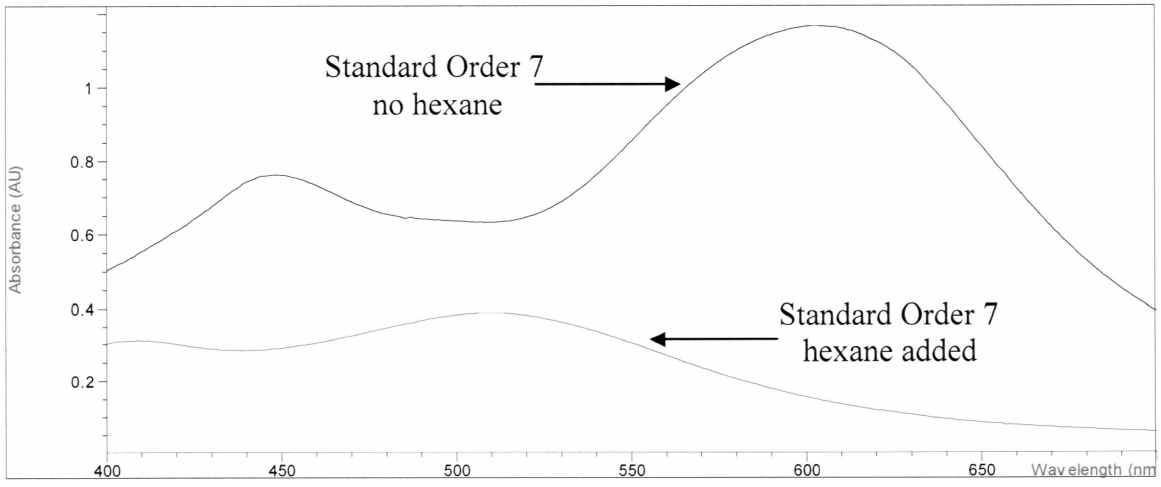

Figure 11: Standard Order 7 after the addition of Hexane.

Figure 11 shows that after the addition of hexane to the silver nanoparticle solution, the absorbance decreased and lambda max shifted toward the blue. This indicated that a surface interaction occurred, which caused a change in the refractive index of the nanoparticle surface. Because of this interaction, the solvent choice was changed from hexane to methanol.

The next series of experiments involved using methanol as the solvent to dissolve the 1-dodecanethiol. The experimental design was similar to that used with hexane, the only difference being that dodecanethiol was present in every preparation at either 1% or 3% molar ratio, (table 6). As in the hexane experiment, four preparations were deoxygenated by purging with $N_2(g)$. Spectra were taken at 1, 2, and 3 hours after addition of dodecanethiol (Figure 12).

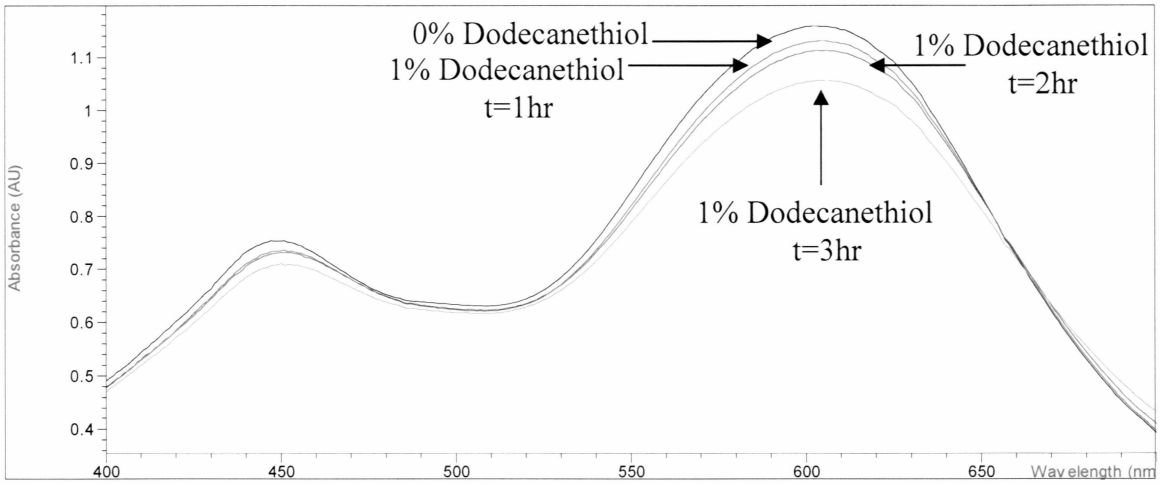

Figure 12: Spectra of Standard Order 6 after addition of 1% Dodecanethiol.

There is a small decrease in the absorbance spectra; however there is not a shift in λ_{max}. This indicates that dodecanethiol has a small effect on the surface of the nanoparticles. This indicates that the hexane used in the first experiment was what was causing such a huge effect. Dodecanethiol itself does not cause much of an effect on the silver nanoparticles.

Carbon Disulfide

When 100μL of 0.004M carbon disulfide was added to silver nanoparticle solutions that were a month old, there was no appreciable change in the absorbance band at lambda max. The second experiment done involved increasing the concentration of carbon disulfide added to the silver nanoparticle solutions. In addition to the old silver nanoparticle solutions, carbon disulfide was added to freshly prepared solutions. As seen in the first experiment, the absorbance at lambda max had a small decrease in intensity. When carbon disulfide was added to freshly prepared solutions that had not been photo-transformed, a change was observed soon after addition. The color of solution changed from a dark yellow to a faint yellow within thirty minutes. After two days, the color was completely gone from these solutions. These changes were tracked using the UV-Vis. After two days the absorbance at lambda max had gone from 0.5 absorbance units to less than 0.1 absorbance units (Figure 13).

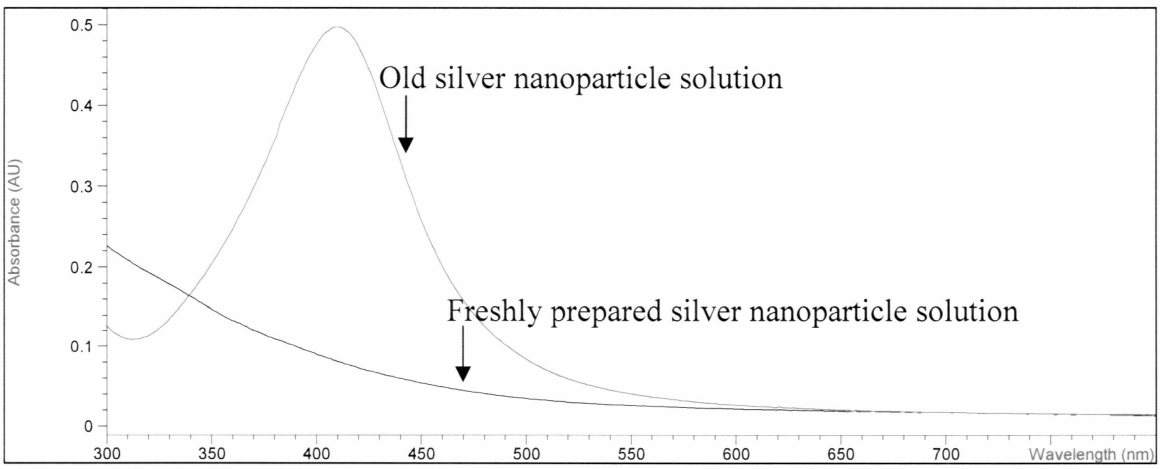

Figure 13: Effects of CS_2 on Old and Freshly Prepared Silver Nanoparticles.

These results suggest that the addition of carbon disulfide disrupts the formation of the silver nanoparticles. The older nanoparticles showed a resistance to the effect of carbon disulfide in solution. These older nanoparticles appear to be stable in solution and the addition of this sulfur containing molecule did not affect the stability of the nanoparticles.

3.3 Oxidation of Silver Nanoparticles in the presence of Cyanide

After the addition of cyanide to the silver nanoparticles, it became apparent that different preparations also have different chemical properties. In the presence of cyanide the silver nanoparticles became more readily oxidizable. Once the cyanide was added, it became apparent that some silver nanoparticle solutions showed a higher resistance to this oxidation effect than others (Figures 14 and 15). Spectra were taken after the initial addition, at 10 minutes, 30 minutes, 1 hour 30 minutes, 1 hour 45 minutes, 1 day, and 3 days after the addition of cyanide. Analysis of data concluded that the reaction occurring was not a 0^{th}, 1^{st}, or 2^{nd} order reaction, nor was it a combination of a fast and slow reaction.

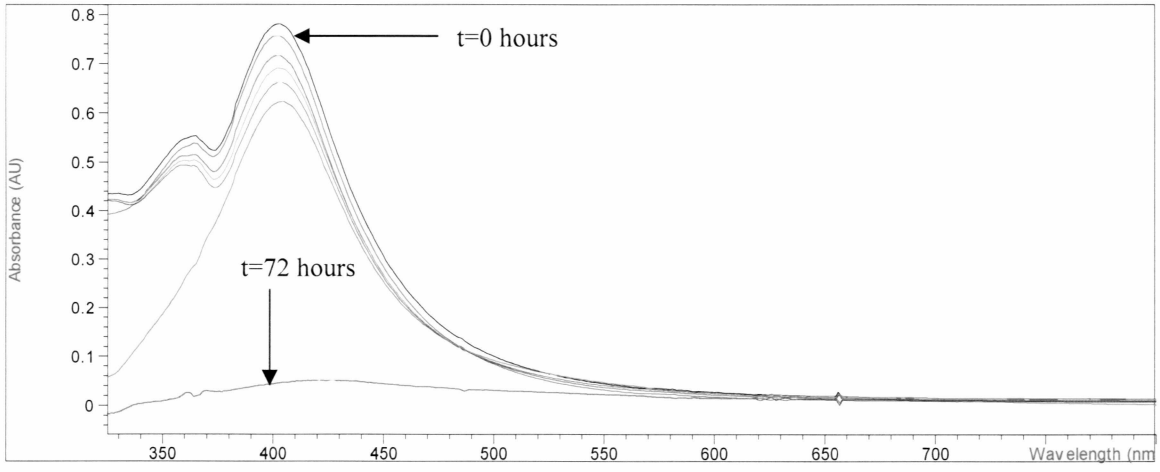

Figure 14: Standard Order 9 over time after addition of 0.1% molar ratio CN to Ag.

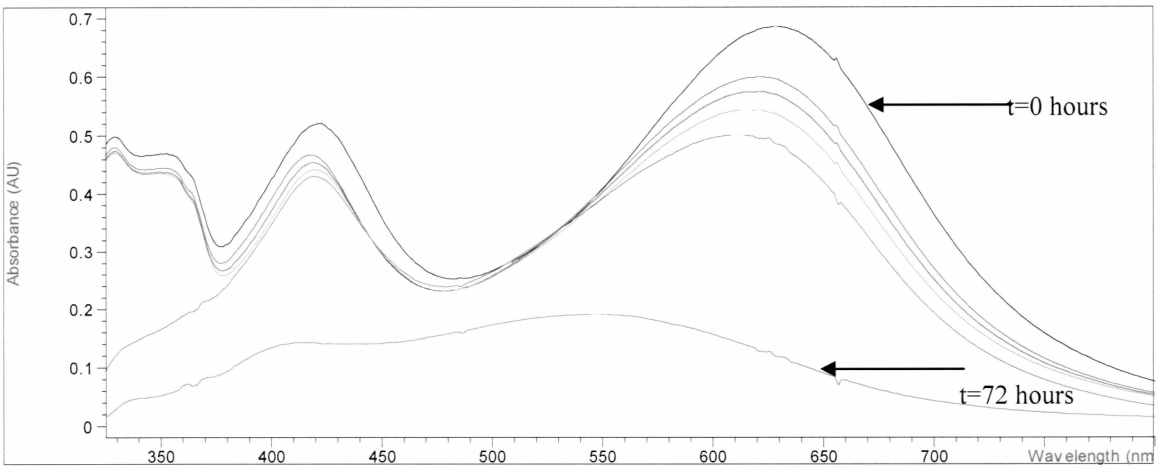

Figure 15: Standard Order 10 over time after addition of 0.1% molar ratio CN to Ag.

It is apparent when looking at these two spectra that standard order 10 showed a higher resistance to oxidation than standard order 9. After 72 hours, standard order 10 still showed an absorbance band, while standard order 9 had none, indicating that it had been completely oxidized. Standard Order 9 and 10 are two representatives on the effects of cyanide on the silver nanoparticle solutions.

Standard orders 2, 6, 7, 8, 11, 12, 13, 14, and 16 showed similar results to standard order 10. The standard order solutions 1, 3, 4, 5, 15 showed results similar to standard order 9. Standard order 9 had an extremely low concentration of citrate, while standard order 10 had an extremely high concentration of citrate. The solutions that showed a similar result as standard order 10 all had moderate to high levels of citrate. Those solutions that were more easily oxidized had low to moderate concentrations of

citrate. This led to the conclusion that the concentration of citrate has an effect on how easily oxidized the silver nanoparticles are in the presence of cyanide.

3.4 Catalytic Properties of Silver Nanoparticles

The catalytic properties of silver nanoparticles were explored by reducing dichlorofluorescein with silver nanoparticles and borohydride. An induction time was observed for each of the solutions (Figure 16).

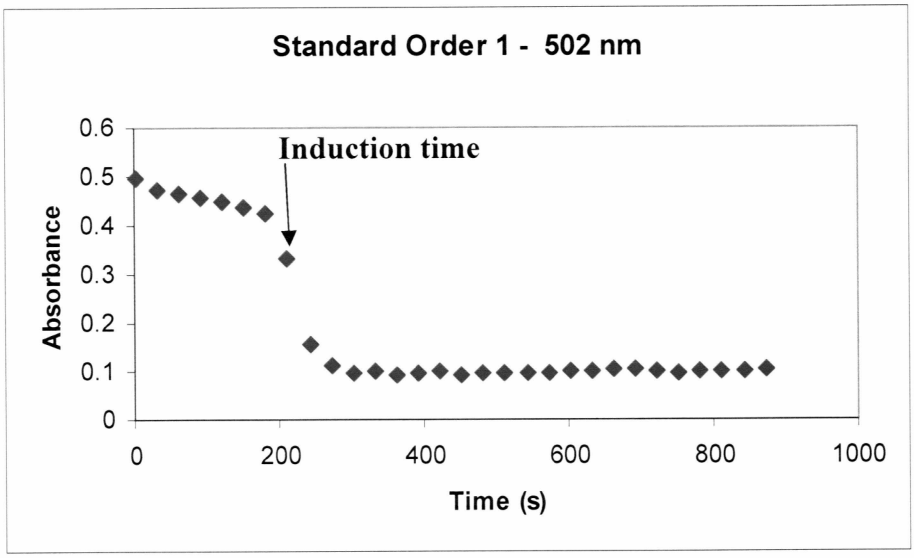

Figure 16: Absorbance versus Time of Standard Order 1 after addition of BH4-.

This indicates the amount of time it takes for the reduction of dichlorofluorescein to begin. The separate preparations of silver nanoparticles showed different induction times (Table 12).

Table 12: Induction Time for the Reduction of Dichlorofluorescein.

Standard Order	Induction Time (s)
1	212
2	602
3	542
4	182
5	900
6	272
7	272
8	182

When standard orders 2, 3, and 5 were used as catalysts the induction time for the dichlorofluorescein was greater than 300 seconds. When standard orders 1, 4, 6, 7, and 8 were used as catalysts the induction time for the reduction of dichlorofluorescein was less than 300 seconds. Standard orders 2, 3, and 5 all had high concentrations of BSPP, while standard orders 1, 6, 7, and 8 all had low concentrations of BSPP. This indicates that the concentration of BSPP could have an effect on the catalytic properties of silver nanoparticles. It was also found that once the induction time began the absorbance band at 500 nm began to decrease in intensity, while an absorbance band at 400 nm began to increase. The band decreasing at 500 nm was the dichlorofluorescein band, while the band increasing at 400 nm was the reduced dichlorofluorescein (Figure 17).

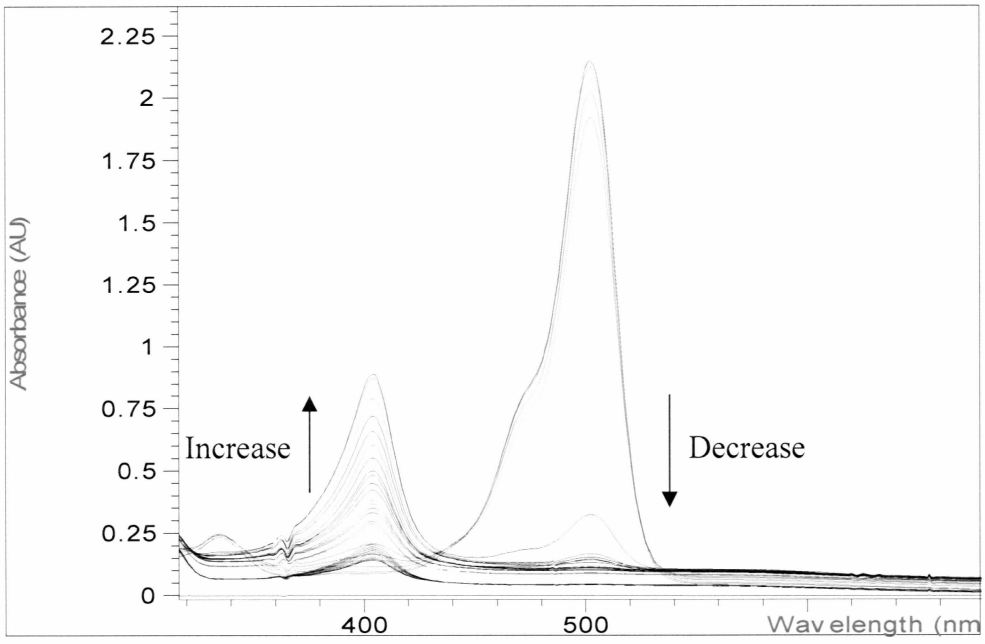

Figure 17: Visible Spectrum of Reducing Dichlorofluorescein using Std Order 6.

3.5 Central Composite Design

The nanoparticles synthesized using the central composite design showed a large variation in colors (Figure 18).

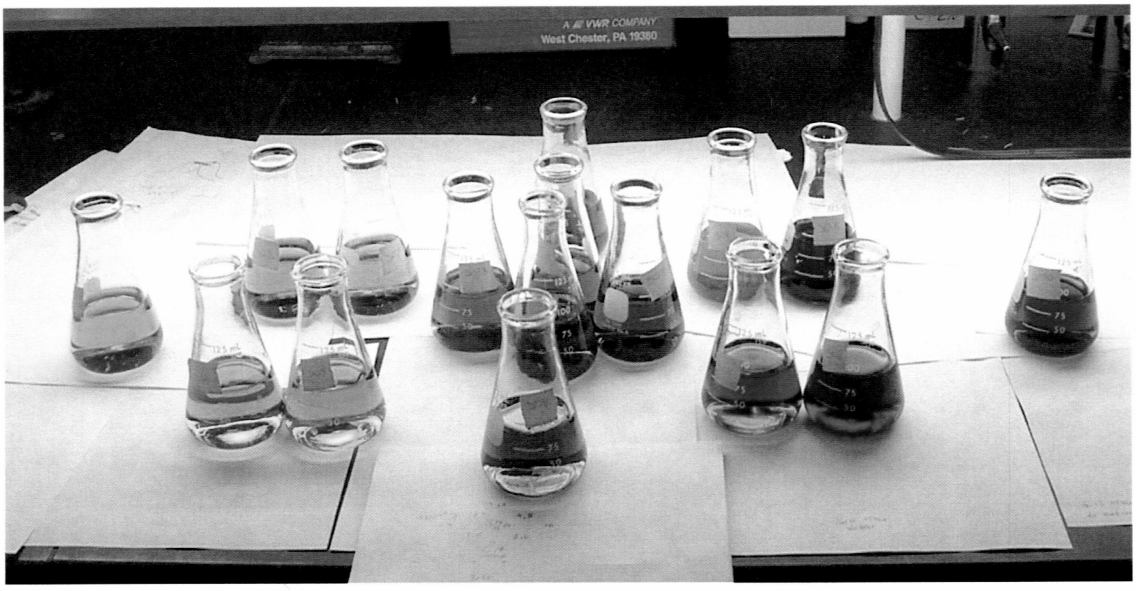

Figure 18: Results from Central Composite Design.

The concentration of citrate increases from left to right, and the concentration of BSPP increases from top to bottom, while the concentration of borohydride increases from left to right in the paired flasks. As citrate increases from left to right, the color of the solutions changes from yellow at low concentrations to green at the median and blue at the high concentrations.

The varying colors of solutions indicate differing sizes and shapes of the nanoparticles. Because there is an apparent gradient of colors as the concentration of

citrate increases, it can be inferred that citrate has an effect on the size and shape of the silver nanoparticles. The concentration of borohydride also appears to have an effect on the size and shape of the silver nanoparticles. In the segmented pairs, there is a visual difference between the low and high concentrations of borohydride. The solutions with the lower concentration of borohydride tend to be a lighter shade of the respective colors than those with a higher concentration. It appears that every reagent used had an important effect on the size and shape of the silver nanoparticles.

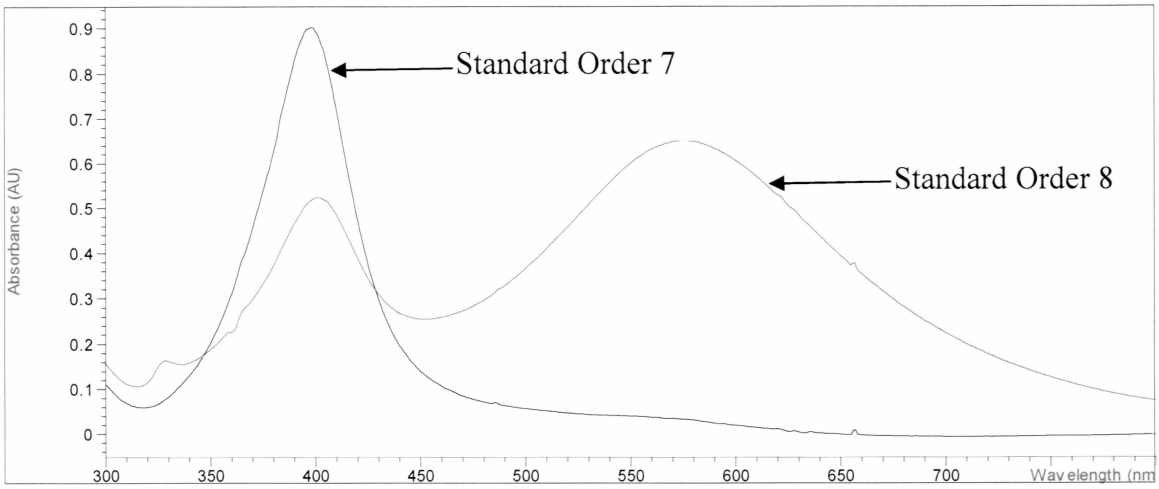

Figure 19: Central Composite Design Standard Order 7 and 8 after 72 hours.

Figure 19 shows standard order 7 and standard order 8 72 hours after preparation. Standard order 7 had a low concentration of citrate, a high concentration of BSPP, and a high concentration of borohydride. Standard order 8 had a high concentration of citrate, a high concentration of BSPP, and a high concentration of borohydride. Standard order 7 had only one absorbance band with an intensity of 0.9, while standard order 8 had two

distinct bands with intensity of 0.5 and 0.6. These results show that the concentration of citrate does have an effect on silver nanoparticle synthesis.

Figure 20 shows standard order 3 and 7. Standard order 3 had a low concentration of citrate, a high concentration of BSPP, and a low concentration of borohydride. Standard Order 7 had a low concentration of citrate, a high concentration of BSPP, and a high concentration of borohydride. When visually inspecting the solutions Standard Order 3 was a green solution, while Standard Order 7 was yellow.

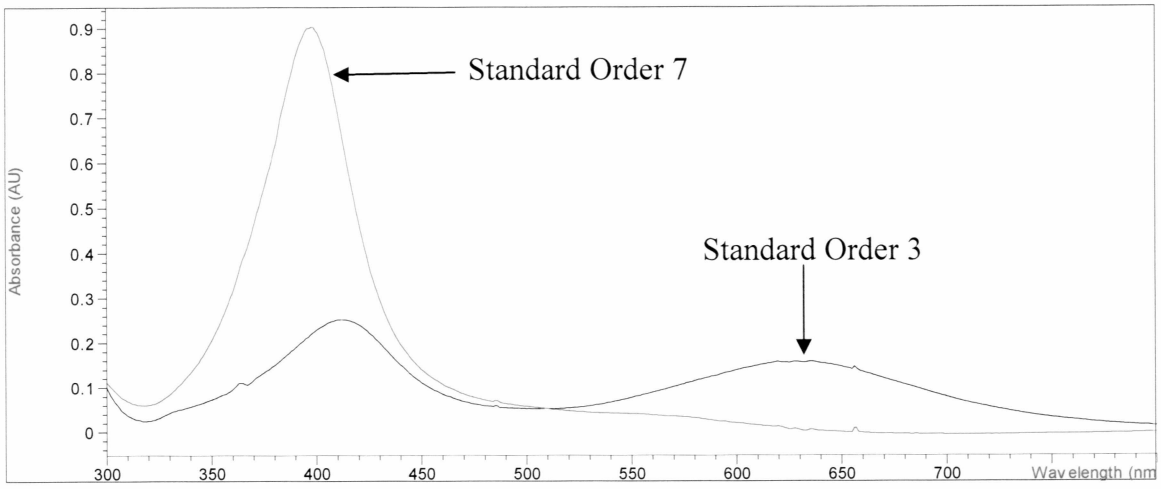

Figure 20: Central Composite Design Standard Order 3 and 7, 72 hrs after preparation.

The spectra show very different absorbance bands. Standard Order 3 has two distinct peaks, while Standard Order 7 has one. The intensity of Standard Order 3 at 2.1 and 1.4 is much less than that of Standard Order 7 which had an absorbance of 0.9. This indicates that the concentration of borohydride also has an effect on the development of silver nanoparticles.

When looking at the effects of BSPP similar results were found (Figure 21).

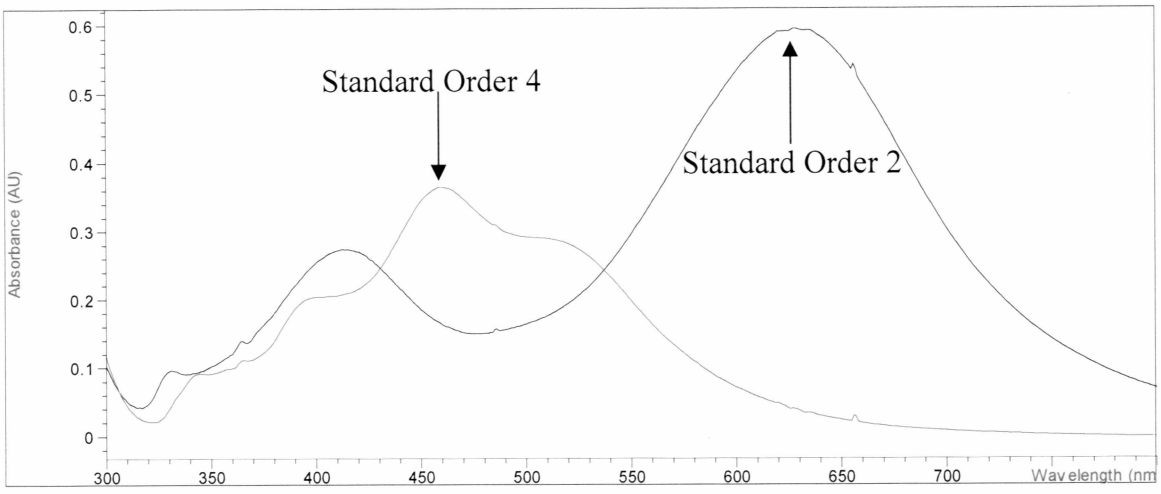

Figure 21: Central Composite Design Standard Order 2 and 4, 72 hrs after preparation.

Figure 21 shows Standard Order 2 and Standard Order 4. Standard Order 2 has a high concentration of citrate, low concentration of BSPP, and low concentration of borohydride. Standard Order 4 had a high concentration of citrate, high concentration of BSPP, and low concentration borohydride. As with the other results, the spectra were different. Standard Order 2 has two distinct absorbance bands while Standard order 4 has one distinct absorbance band. Standard Order 2 had an intensity of 0.26 and 0.59 while the intensity of Standard Order 4 was at 0.36. The difference in the absorbance spectra indicate that BSPP also has an effect on the synthesis of silver nanoparticles.

3.6 Nanoparticle Sizing

The Brookhaven 90 Plus instrument measured the size of the nanoparticles at the time intervals of 9, 10, and 11 hours after the photo induction process began (Table 13).

Table 13: Size of Nanoparticles in Solution.

Standard Order	Size after 8 hours (nm)	Size after 9 hours (nm)	Size after 10 hours (nm)	Size after 11 hours (nm)	Average Size (nm)	Difference in Size (nm)
1	41.2	39.5	40.1	48.9	42.43	+7.7
2	38.2	34.0	30.2	29.8	33.05	-8.4
3	43.6	40.5	39.0	38.2	40.33	-5.4
4	26.6	25.2	26.1	25.9	25.95	-0.7
5	Not available	57.9	48.5	37.6	48.00	-20.3
6	54.0	42.2	41.4	36.7	43.58	-17.3
7	26.4	26.3	26.0	26.3	26.40	-0.1
8	63.3	44.7	42.4	39.7	47.53	-23.6

Concentration of reactants from table 4.

There was no measurement given of standard order 5 at 8 hours due to hydrogen gas production from the oxidation of borohydride interfering with the light source on the instrument.

The mean values show that the average size of the nanoparticles from 8 to 11 hours after photo transformation began ranged from 25.95nm to 48nm. There does not appear to be any systematic behavior when considering the concentration of reactants used and the average size of the nanoparticles. When analyzing the size data graphically

(Figure 22) it became apparent that four nanoparticle solutions showed a significant decrease in size compared to the other four nanoparticle solutions.

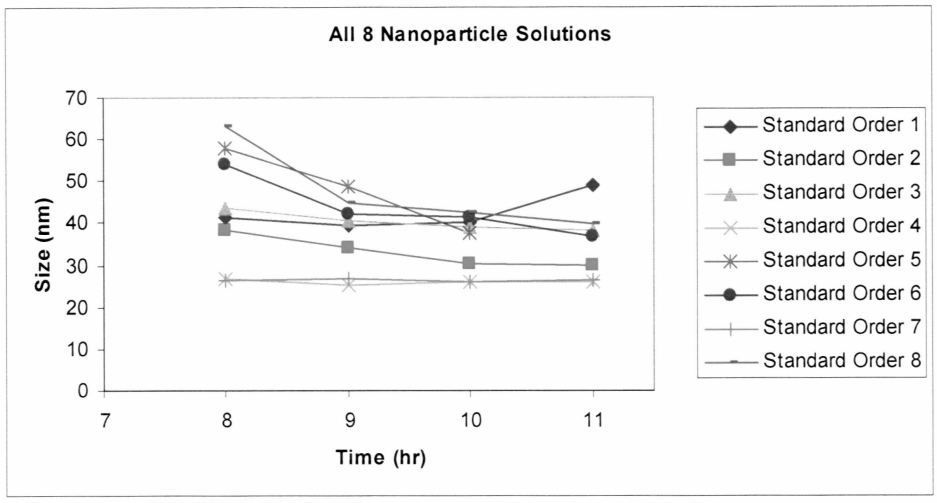

Figure 22: All Nanoparticle Solutions over Time.

Standard Orders 2, 3, 5, 6, and 8 all showed a significant decrease in size over time (Figure 23).

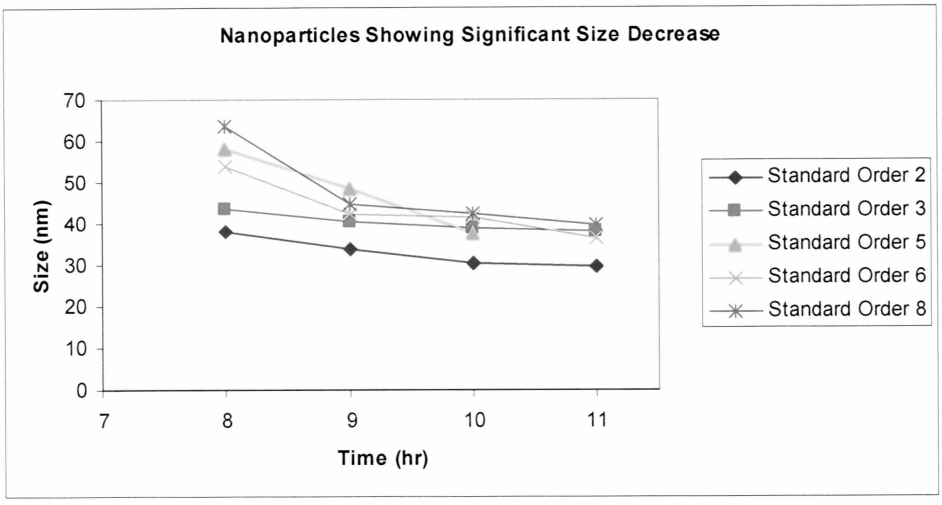

Figure 23: Standard Orders 2, 3, 5, 6, and 8 over Time.

The preparation for these nanoparticle solutions showed no relationship. The three solutions that showed a large change in size from 8 to 11 hours were Standard Orders 5, 6, and 8. All three of these solutions had high concentration of sodium borohydride. The only preparation that showed a growth in nanoparticle size rather than a decrease was Standard Order 1. This particular preparation had low concentration of each reagent.

Chapter 4

Conclusions

Silver nanoparticles have interesting optical properties that make them likely candidates to be used as a part of sensor systems. Syntheses varying the concentration of the reactants (citrate, BSPP, and borohydride) produced nanoparticles with varying shapes and sizes with varying chemical and optical properties. These chemical and optical properties were investigated during various treatments.

Four separate syntheses were done over the course of two years which produced solutions with varying λ_{max} and intensity. A central composite design experiment was performed to determine which reactant had the largest effect. It was found that each reactant was integral in the development of silver nanoparticles.

Surface interactions of sulfur containing molecules were investigated with L-cysteine, 1-dodecanethiol, and carbon disulfide. The initial experiments involving cysteine found that when 25% molar ratio cysteine to silver was added to a silver nanoparticle solution, there was a large blue shift in the absorbance at λ_{max}, and with subsequent additions of cysteine showing small shifts in the absorbance band. Subsequent experiments systematically decreased the molar ratio until a photometric titration was performed with a range of 0 to 5% molar ratio cysteine to silver. Solutions with varying preparations displayed different results. Some nanoparticle solutions showed as much as a 44nm shift in λ_{max} while other preparations showed no net change. Those nanoparticle solutions synthesized with a high concentration of citrate showed a

smaller shift in λ_{max} (less than 10 nm shift) than those synthesized with lower concentrations of citrate. This likely occurs because the adsorbate layer on the silver nanoparticles is changing with the addition of cysteine. This would change the local refractive index which would cause a change in the absorbance at lambda max. The particular composition of the adsorbate layer is determined by the synthesis.

The second experiment involving sulfur containing molecules involved the addition of 1-dodecanethiol. It was found that with the addition of 1 and 3% molar ratio dodecanethiol to silver, there was a slight decrease in intensity but no change in λ_{max}. From this experiment it was discovered that the solvent hexane has a surface interaction with silver nanoparticles and the silver nanoparticle solutions became completely decolorized in the presence of hexane. It can be inferred that the hexane has an affinity for the interface between the solid silver nanoparticles and water. This would result in the citrate and BSPP anions of the electrostatic double layer being displaced from the silver and the agglomeration of silver would occur.

When carbon disulfide was added to nanoparticle solutions that were developed a month prior, there was no dramatic change in the absorbance band. However, when carbon disulfide was added to freshly prepared silver nanoparticle solutions, the absorbance band disappeared. This indicates that in the presence of carbon disulfide, older nanoparticles are stable while the development is disrupted in freshly prepared solutions.

In another series of experiments, the silver nanoparticles synthesized via the central composite design were exposed to cyanide. Silver nanoparticles become more

readily oxidizable by O_2 in the presence of cyanide. Those nanoparticle syntheses that had a high concentration of citrate showed a higher resistance to oxidation than those that were prepared with lower concentrations of citrate. Josh Klynstra performed an experiment in which he discovered that nanoparticles with a smaller diameter are more easily oxidized than those with a larger diameter. From this finding it can be inferred that those nanoparticles that were synthesized with a higher concentration of citrate had a larger diameter than those with a lower concentration of citrate, which would give them a higher resistance to oxidation in the presence of cyanide.

The catalytic properties of silver nanoparticles were also investigated by using them as a catalyst for the reduction of dichlorofluorescein. It was found that there was an induction time in the reduction of dichlorofluorescein. This induction time was different with the different preparations of silver nanoparticles. Those solutions that had a low concentration of BSPP had an induction time of less than 300 seconds. Those solutions with a high concentration of BSPP typically had induction times greater than 300 seconds. It was also found that an absorbance band at 400nm appeared over time as the absorbance band at 500nm disappeared. The 400nm band appearing was determined to be the reduced dichlorofluorescein band while the 500nm band was the dichlorofluorescein band.

The final experiment done tracked the size development of the nanoparticles over a time range of 8 to 11 hours. It was found that the nanoparticles averaged in size from 25.95nm to 48nm. There does not appear to be any systematic effect of concentration of reactants and the size of the nanoparticles. It was observed that those solutions

containing nanoparticles that changed in size considerably over time all had a high concentration of borohydride. It was found that the nanoparticles typically decreased in size over time.

The series of experiments done over the course of two years shows that different preparations do in fact display different chemical properties when exposed to different chemical treatments. These properties could lead to the discovery of more sensitive sensors which could decrease the limit of detection when used as part of a sensor system.

References

1. Jin R.; Coa Y. W.; Mirkin C. A.; Kelly K. L.; Schatz G. C.; Zheng J. G. *Science* **2001**, *294*, 1901-1903.
2. Schmid, G.; *Clusters and Colloids – From Theory to Applications*; VCH: New York. 1994.
3. Nath N.; Chilkoti A. *Anal. Chem.* **2002**, *74*, 504-509.
4. Lyon L. A.; Musick M. D.; Natan M. J. *Anal.Chem.* **1998**, *70*, 5177-5183.
5. Mclaughlin C.; MacMillan D.; McCardle C.; Smith E. *Anal. Chem.* **2002**, *74*, 3160-3167.
6. Wang J.; Li J.; Baca A. J.; Hu J.; Zhou F.; Yan W.; Pang D. *Anal. Chem.* **2003**, *75*, 3941-3945.
7. Nimeyer C. M.; Adler M.; Pignataro B.; Lenhert S.; Gao S.; Chi L.; Fuchs H.; Blohm D. *Nucl. Acids. Res.* **1999**, *27*, 4553-4561.
8. Lin S. Y.; Liu S. W.; Lin C. M.; Chen C. H. *Anal. Chem.* **2002**, *74*, 330-335.
9. Pillai Z. S.; Kamat P. V. *J. Phys. Chem. B.* **2004**, *108*, 945-951.
10. Chen S.; Carroll D. L. *Nano Letters* **2002**, *2*, 1003-1007.
11. Callegari A.; Tonti D.; Chergui M. *Nano Letters* **2003**, *3*, 1565-1568.
12. Mock J. J.; Barbic M.; Smith D. R.; Schultz D. A.; Schultz S. *J. Chem. Phys.* **2002**, *116*, 6755-6759.
13. Wang W.; Chen X.; Efrima S. *J. Phys. Chem. B.* **1999**, *103*, 7239-7246.

14. Haes A. J.; Zou S.; Schatz G. C.; Van Duyne R. P. *J. Phys. Chem. B.* **2004**, *108*, 109-116.

15. Kapoor S. *Langmuir* **1998**, *14*, 1021-1025.

16. Porter, Jr. L. A.; Ji D.; Westcott S. L.; Graupe M.; Czernuszewicz R. S.; Halas N. J.; Le T. R. *Langmuir* 1998, *14*, 7378-7386.

17. Jiang X.; Xie Y.; Lu J.; Zhu L.; He W.; Qian Y. *Langmuir* **2001**, *17*, 3795-3799.

18. Riboh, J. C.; Haes, A. J.; McFarland, A.D.; Yonzon, C. R.; Van Duyne, R. P. *J. Phys. Chem. B* . **2003**, *107*, 1772-1780.

19. Haes, A. J.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2002**, *124*, 10596-10604.

20. Mulvaney P.; Linnert T.; Henglein A. *J. Phys. Chem. B.* **1991**, *95*, 7842-7846.

21. Finsey R. *Adv. Colloid Inter. Sci.* **1994**, *52*, 79-143.